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Correlations between ecdysteroid titers and integument structure in nymphs of the tick, *Amblyomma hebraeum* Koch (Acarina: Ixodidae)

by

P. A. DIEHL *, J. E. GERMOND * and M. MORICI *

With 13 figures and 1 table

Abstract

The temporal correlation between the ecdysteroid titer and the structure of the extensible integument was studied during the development of nymphs to adults in the ixodid tick *Amblyomma hebraeum* Koch. Using a radioimmunoassay (RIA) only small hormone concentrations were found in hemolymph and in whole ticks during the first 17 days after begin of feeding. During this phase, much new endocuticle was deposited and the mitotic period initiated. After the 17th day the ecdysteroid titer increased to a maximum at day 23 (about 14 ng ecdysterone equivalents per tick). During this phase the mitotic period ended, apolysis was initiated, and the tick began to deposit the epicuticle (day 23). Thereafter the titer dropped to low values around ecdysis (day 31-34; about ½ ng per tick). During this time the epicuticle (day 23-25) and the exocuticle (day 25-ecdysis) were deposited and the nymphal cuticle digested.

Chemical analysis by high performance liquid chromatography and RIA showed the presence of ecdysone and mainly ecdysterone.

INTRODUCTION

Reports on the presence of molting hormones in arthropods other than crustaceans and insects are still very rare. Ecdysteroids have been demonstrated in the myriapods *Hanseniella ivoriensis* (Juberthie-Jupeau *et al.* 1979) and *Lithobius forficatus* (Joly

* Institute of Zoology, University, Chantemerle 22, CH-2000 Neuchâtel, Switzerland.

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et al. 1979), in the xiphosuran *Limulus polyphemus* (WINGET & HERMAN 1976), and in the spiders *Pisaura mirabilis* (BONARIC & DE REGGI 1977) and *Opilio ravennae* (ROMER & GNATZY 1981).

In order to elucidate the possible involvement of ecdysteroids in the control of tick molting, we began a series of investigations in the two ticks, *Ornithodoros moubata* and *Amblyomma hebraeum*. Chemical analysis indicated the presence of ecdysone and ecdysterone in the final nymphal stage of *O. moubata* (GERMOND *et al.* 1982). Molting hormone activity was also demonstrated in the hemolymph of *Ornithodoros porcinus porcinus* using the *Musca* bioassay (MANGO & MOREKA 1979). These two *Ornithodoros* species belong to the family Argasidae which generally feed rapidly, have more than two molts before reaching the adult stage, and females show several gonotrophic cycles.

By chemical analysis we could reveal the presence of ecdysone and ecdysterone also in nymphs of the tick *Amblyomma hebraeum* (DELBECQUE *et al.* 1978). In contrast to *Ornithodoros*, this tick belongs to the family Ixodidae which is biologically quite different from the Argasidae. Ixodids feed slowly, have 2 molts during postembryonic development, and the female has only 1 gonotrophic cycle. Here, we will report on the temporal correlation between the integument structure and the ecdysteroid concentration in *Amblyomma hebraeum* during its development from the nymphal to the adult stage.

MATERIAL AND METHODS

Animals: Nymphs of *Amblyomma hebraeum* were fed on white rabbits for the collection of ticks of various engorgement states. Large numbers of fully engorged ticks fed on cows were kindly provided by CIBA-GEIGY Ltd. (Les Barges). The fed ticks were kept in several tubes, each containing a few animals (darkness, 26° C, about 90% relative humidity). When necessary, a tube was removed and the ticks extracted. This procedure ensures a good synchronization of the molting processes. Indeed, if frequently disturbed, the nymph may delay molting or may not molt at all. Ecdysis occurred during day 31-34 after beginning of feeding.

Methods: The methods used for the cytology of the integument (alloscutum, extensible cuticle), for the hormone extraction, the analysis by high-performance liquid chromatography (HPLC) and the radioimmunoassay (RIA) are described by GERMOND *et al.* (1982). Hemolymph was obtained by making small incisions with a sharp razor blade at the base of the coxae or mouthparts, or into the body integument. Care was taken to avoid puncturing of the midgut. However, between day 19 after beginning of feeding and the day of molting, hemolymph was always contaminated with some molting fluid which is present in copious amounts throughout this period.

RESULTS

Correlations between ecdysteroid titer and structure of the integument

As in other ticks, a bloodmeal is necessary for the induction of the molting processes in the nymphs of *Amblyomma hebraeum*. Feeding in this tick lasts for about 7 days. At 26° C molting to adults occurs about 31-34 days after the beginning of feeding.

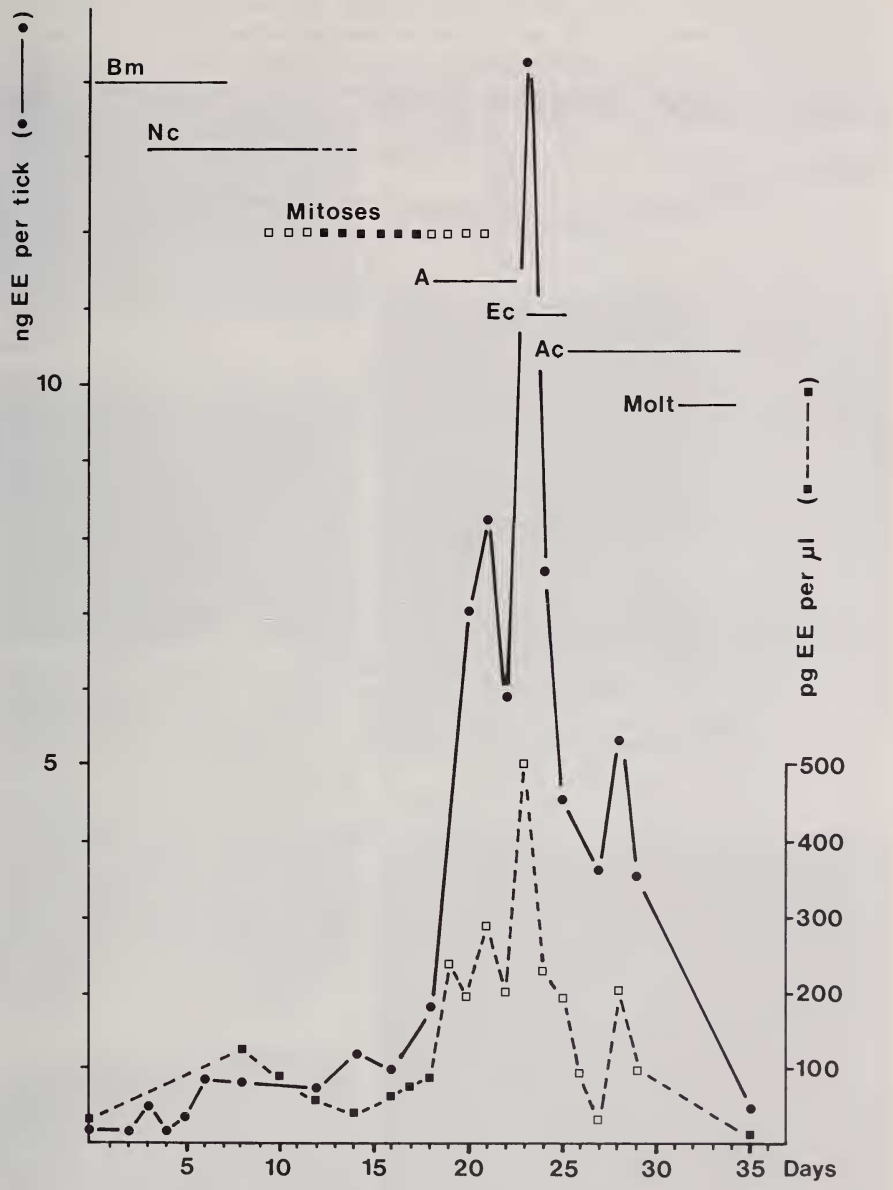


FIG. 1.

Temporal correlation between integument structure and ecdysteroid titer during the development of nymphs to adults in the tick *Amblyomma hebraeum*. The ecdysteroid titer determined by the RIA (mean of duplicates) is expressed as ng ecdysterone-equivalents (=EE) per tick (●——●) or pg EE per µl hemolymph (■- - - -■: hemolymph; □- - - -□: hemolymph diluted with molting fluid) as a function of days after beginning of feeding. Bm: bloodmeal. Nc: deposition of nymphal endocuticle during and after the meal. Mitoses: □ few; ■: many. A: apolysis. Ec: epicuticle deposition. Ac: production of adult exocuticle (preecdysial cuticle). Molt: between day 31—34.

During the first 16 days following the beginning of the meal, only little radioimmunoassay (RIA)-positive material was detected in whole nymphs (about 0.5-1 ng ecdysterone-equivalents (= EE) per tick) or in hemolymph (50-120 pg EE/µl; see Fig. 1). During this time the hypodermis deposited much endocuticle up to day 12-14 (see Figs. 1-3). The nymphal cuticle thus became thick and mecanically very resistant. The mitotic period was initiated around day 9. Intense mitotic activity was observed between day 12 and 17. Towards day 13-15 the ticks became also less mobile; after day 16 no leg movements were observed.

After the 17th day the ecdysteroid titer in whole animals began to rise and reached highest values around day 23 (about 14 ng EE/tick). The hemolymph titer appeared also to rise to a maximum around day 23. It must be remembered, however, that during this period the hemolymph samples got contaminated with molting fluid. The true concentrations are certainly higher, and the hemolymph titer curve must therefore be interpreted with caution.

During this period of rising ecdysteroid concentration (day 17-23), the mitotic phase ended and the hypodermis underwent apolysis (Figs. 1, 3). The highest hormone titer of about 14 ng/tick was detected when the hypodermis began to deposit the adult epicuticle (day 23; Figs. 1, 4).

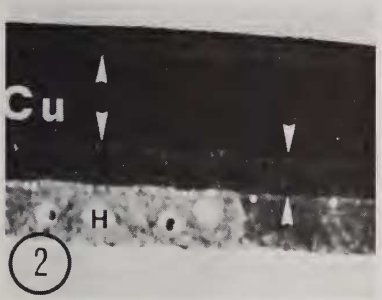
After day 23 up to day 25 the hormone concentration dropped rather steeply (Fig. 1). This was temporarily correlated with the deposition of the epicuticle (Figs. 4, 5, 8-10). As in other ixodid ticks, the highly folded epicuticle from the extensible cuticle in *A. hebraeum* females consists of an outer epicuticle, a cuticulin layer and a dense layer. Electron-dense epicuticular filaments traverse the epicuticle (Figs. 9-12). These flat, twisted ribbon-like structures appear to be in physical continuity with the cuticulin (Figs. 9, 10) and with electron-dense material near or at the surface of the microvilli (Fig. 9). Smaller dense filaments are present in the outer half of the epicuticle (Fig. 11). A tubular network can be observed near the border with the future procuticle (Fig. 12).

After day 25 up to ecdysis around day 31-34 the ecdysteroid titer continued to drop and reached again low values of about 0.5 ng EE/tick and 20 pg EE/µl hemolymph in freshly molted females. During this period the future adult exocuticle containing many pore canals and few dermal gland canals was deposited, and the nymphal cuticle partially digested (Figs. 6, 7, 13). After ecdysis endocuticle deposition continued for several days. In contrast to the homogeneous appearance of the exo- and endocuticle, the last endocuticle layer present in hungry females has a zig-zag-like arrangement (Fig. 13).

FIGS. 2—7.

Changes in integument structure during nymphal-adult development. Semithin sections. H:hypodermis. CU: cuticle. Magnification: 330 x.

FIG. 2. Day 9 after beginning of feeding. Endocuticle deposited during feeding: ◀ ▶, and after feeding: ▶ ◀. — FIG. 3. Day 20. Apolysis, with creation of an exuvial space: ✻. Endocuticle deposited after feeding: ▶ ◀. — FIG. 4. Day 23. Beginning of epicuticle (▬▶) deposition in female extensible cuticle. Dermal glands: ▶. — FIG. 5. Day 26. Epicuticle fully formed. — FIG. 6. Day 30, just before ecdysis. Female extensible cuticle below the remainder of the digested nymphal cuticle (NC) and the molting membrane (MM). Exocuticle deposited. Dermal gland (▶) with duct (▬▶). — FIG. 7. Extensible cuticle from hungry female. Border between exo- and endocuticle not visible. Dermal glands (▶). Dermal gland duct (▬▶).



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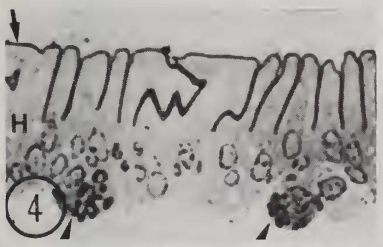

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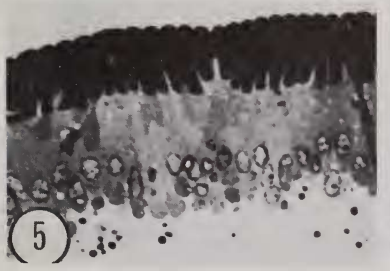

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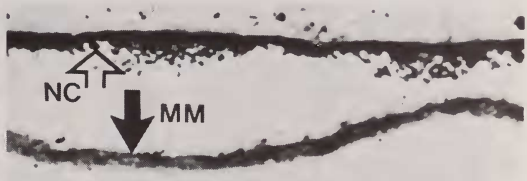

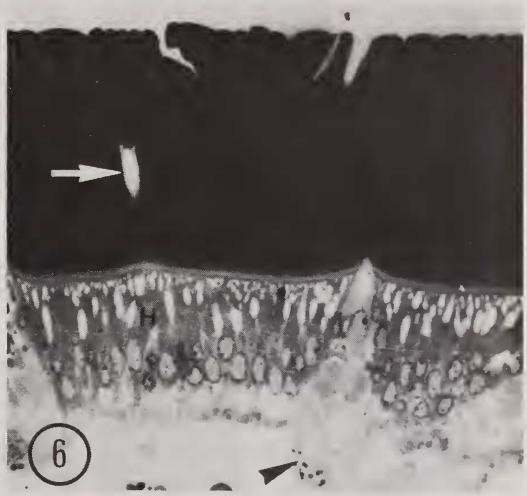

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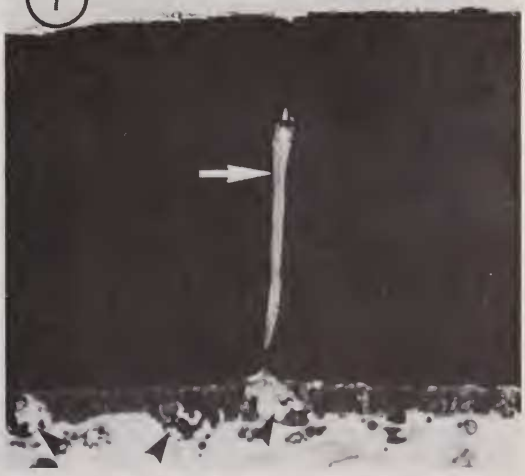

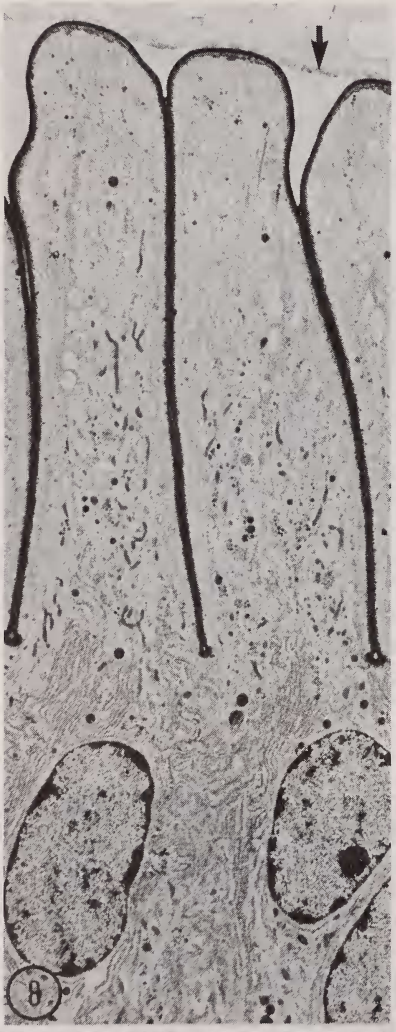
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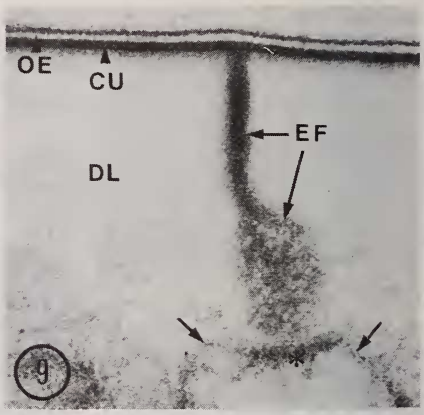
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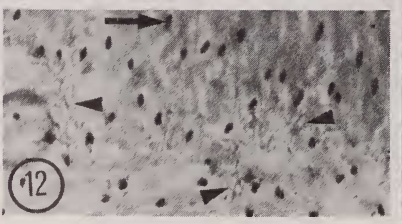
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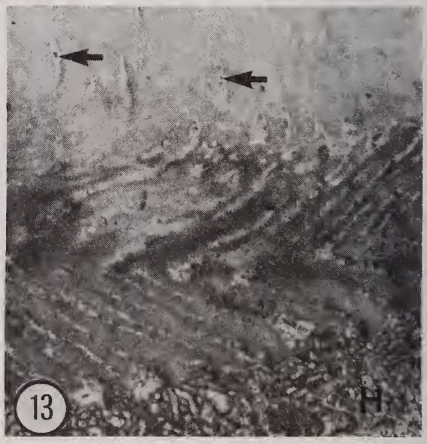
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Chemistry of the ecdysteroids

By combined gas chromatography — mass spectrometry we have already demonstrated the presence of ecdysone and ecdysterone in whole nymphs at the moment of highest ecdysteroid concentration (see DELBECQUE *et al.* 1978). Using high-performance liquid chromatography (HPLC) and the radioimmunoassay (RIA) as detection method, we could determine that at several development stages nearly all of the RIA-positive material in the hemolymph and in whole ticks migrated like ecdysone and ecdysterone, respectively. Ecdysterone was always the dominating ecdysteroid. However, comparatively less ecdysterone was present in hemolymph (ecdysterone-ecdysone ratio 2,7 : 1-7,7 : 1) than in whole ticks (9,3 : 1-17,5 : 1). This ratio was at its extreme at the time of highest hormone concentration (Table 1).

TABLE 1.

Ratio ecdysterone : ecdysone (weight/weight) in hemolymph and whole nymphs at different stages after beginning of feeding. The samples were separated by HPLC (reverse mode RP-18) and the ecdysteroid content of the fractions assayed by RIA (for details see Germond et al. 1982).

Sample	Ratio ecdysterone: ecdysone
Hemolymph	
days 19-21 (pooled)	4 : 1
day 23	7,7 : 1
days 24-26 (pooled)	2,7 : 1
whole nymphs	
days 12, 14, 16 (pooled)	12,4 : 1
day 20	10 : 1
day 23	17,5 : 1
days 27 & 29 (pooled)	9,3 : 1

In whole animals, very polar RIA-positive material was detected near ecdysis (day 27-29). However, its chemical nature is not yet explored.

FIGS. 8—13.

Ultrastructure of female extensible cuticle.

FIG. 8. Day 25. Beginning of epicuticle deposition. Molting membrane (➡). Magnification: 2·700 x. — FIG. 9. Day 25. Outer epicuticle (OE). Cuticulin layer (CU). Dense layer (DL). Epicuticular filament (EF) in relation with an electron-dense material (plaque: ✻) near or in the cell membrane (➡) at the tip of a microvillus. Magnification: 168·000 x. — FIG. 10. Day 25. Outer epicuticle not yet formed. Epicuticular filament in intimate contact (➡) with the cuticulin layer. Magnification: 168·000 x. — FIG. 11. Day 28. Presence of dense filaments (▶) in the dense layer. Epicuticular filaments (➡). — Magnification: 39·000 x. — FIG. 12. Day 28. Faint tubular network (▶) near the border epicuticle-exocuticle. Epicuticular filaments (➡). Magnification: 11·700 x. — FIG. 13. Hungry female. The innermost part of the endocuticle shows a zig-zag-like arrangement. Pore canals (➡). Hypodermis: H. Magnification: 8·100 x.

DISCUSSION

Integument structure

From our cytological studies it appears that the structure of the extensible cuticle (alloscutum) from female *Amblyomma hebraeum* resembles strongly the one from *Boophilus microplus* (Filshie 1976; Hackman 1982) or from *Hyalomma asiaticum* (Amasova 1975). However, the regularly striated substructure in the outer epicuticle described for *B. microplus* (Filshie 1976) seems to be absent in *A. hebraeum*.

The cuticulin layer is in intimate contact with the epicuticular filaments. In view of the comparable electron-density and thickness one may even speculate that both structures are made up of the same material. By this way growth of the cuticulin layer may occur. Indeed, as observed in *B. microplus* by Filshie (1976), considerable increase in epicuticle surface is observed even after fusion of the cuticulin patches into a continuous layer. However, alternatively the filaments may convey cuticulin precursors from the cell surface to the cuticulin layer, as suggested by Filshie (1976). In addition, they may also be responsible for the transport of surface lipids to the outside of the cuticle.

The function of the dense filaments remains obscure. They seem to end very near the cuticulin layer or even to be in continuity with this layer. These filaments may also convey substances to the outside of the cuticle or help to anchor the cuticulin layer to the dense layer.

In contrast to *B. microplus* (Filshie 1976) the exocuticle of the alloscutum in *A. hebraeum* has a homogeneous aspect and shows no apparent lamellation.

Correlations between the ecdysteroid titer and the integument structure

The deposition of much nymphal endocuticle during and after feeding and the initiation of cell multiplications took place during low ecdysteroid concentrations. Cessation of cell division and apolysis occured during rising hormone titers. This is comparable with the situation in the argasid tick *O. moubata*, except for the deposition of endocuticle which is limited to a few lamellae only (Germond *et al.* 1982).

Several reports show that apolysis apparently occurs concomitantly with elevated ecdysteroid titers (e.g. *Calpodes* (Dean *et al.* 1980); *Manduca* (Riddiford 1980; Wielgus *et al.* 1979); *Tenebrio* prenymphs (Connat *et al.* in preparation) and nymphs (Delachambre *et al.* 1980)). This seems to be corroborated also by *in vitro* results. Apolysis can be induced by ecdysteroids in cultures of insect integument (e.g. Agui 1977) or of crustacean integument (Freeman & Costlow 1979).

It is generally admitted that the large ecdysteroid peak is responsible for the initiation and synchronisation of cuticle deposition. Epicuticle synthesis is concomitant with highest hormone concentration e.g. in *Tenebrio* (Delachambre *et al.* 1980), in the myriapod *Hanseniella* (Juberthie-Jupeau & Juberthie 1980), in the argasid tick *O. moubata* (Germond *et al.* 1982) and in the ixodid tick *A. hebraeum* (this report).

As in insects or in the tick *O. moubata*, procuticle deposition and digestion of the nymphal cuticle in *A. hebraeum* does apparently not require high ecdysteroid titers.

Chemistry of the ecdysteroids

According to this study most of the RIA-positive material migrated in HPLC like ecdysone and ecdysterone, respectively. This is confirmed by our earlier results using gas chromatography-mass spectrometry (Delbecque *et al.* 1978).

Ecdysterone appears to be the principal ecdysteroid involved in the control of the molting processes in crustaceans and insects (e.g. *Calpodes* (DEAN *et al.* 1980), *Orconectes* (KELLER & SCHMID 1979), *Tenebrio* (DELBECQUE *et al.* 1975)), but also in the ticks *O. moubata* (GERMOND *et al.* 1982) and *A. hebraeum*. According to preliminary chemical identification by TLC, ecdysterone also appears to be the principal hormone in the myriapod *Hanseniella* (JUBERTHIE-JUPEAU *et al.* 1979) and in the spider *Pisaura* (BONARIC & DE REGGI 1977). Thus the prevalence of ecdysterone for molting control seems to be common amongst arthropods.

We noticed the presence of RIA-positive material appearing towards the end of the instar. We have not yet analysed the chemistry of these polar products. They may represent hormone inactivation products.

Our results in nymphs of *A. hebraeum* and *O. moubata* (GERMOND *et al.* 1982) demonstrate the presence of ecdysone and ecdysterone — the principal molting hormones of insects and crustaceans — in the two tick families. In addition, we can conclude that the temporal correlation between the ecdysteroid titer and the integument structure is comparable to what is known in insects and crustaceans. These results, together with the observations that exogenous molting hormones can induce supermolting in adult argasid ticks (KITAOKA 1972; MANGO *et al.* 1976) demonstrate the importance of ecdysteroids in the control of tick molting processes.

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